Long-term cardiovascular effects of neonatal dexamethasone treatment: hemodynamic follow-up by left ventricular pressure-volume loops in rats

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Dexamethasone is clinically applied in preterm infants to treat or prevent chronic lung disease. However, concern has emerged about adverse side effects. The cardiovascular short-term side effects of neonatal dexamethasone treatment are well documented, but long-term consequences are unknown. Previous studies showed suppressed mitosis during dexamethasone treatment, leading to reduced ventricular weight, depressed systolic function, and compensatory dilatation in prepubertal rats. In addition, recent data indicated a reduced life expectancy. Therefore, we investigated the long-term effects of neonatal dexamethasone treatment on cardiovascular function. Neonatal rats were treated with dexamethasone or received saline. Cardiac function was determined in 8-, 50-, and 80-wk-old animals, representing young adult, middle-aged, and elderly stages. A pressure-conductance catheter was introduced into the left ventricle to measure pressure-volume loops. Subsequently, the hearts were collected for histological examination. Our results showed reduced ventricular and body weights in dexamethasone-treated rats at 8 and 80 wk, but not at 50 wk. Cardiac output and diastolic function were unchanged, but systolic function was depressed at 50 and 80 wk, evidenced by reduced ejection fractions and rightward shifts of the end-systolic pressure-volume relationships. We concluded that previously demonstrated early adverse effects of neonatal dexamethasone treatment are transient but that reduced ventricular weight and systolic dysfunction become manifest again in elderly rats. Presumably, cellular hypertrophy initially compensates for the dexamethasone treatment-induced lower number of cardiomyocytes, but this mechanism falls short at a later stage, leading to systolic dysfunction. If applicable to humans, cardiac screening of a relatively large patient group to enable secondary prevention may be indicated.

METHODS

Animals

Pregnant Wistar rats (270–300 g) were housed individually and kept under conventional housing conditions. Pups were born on day 21 or 22 of gestation. On the day of birth, male pups were selected and randomly divided into treatment and control groups. Treatment and control animals were kept separately and placed with foster mothers in groups of four to six pups. Rat pups in the treatment group were injected intraperitoneally with dexamethasone (Organon, Oss, The Netherlands), with a 3-day tapering dose following a protocol used before (19). Consequently, the treated animals received 0.5, 0.3, and 0.1 μg/g body wt dexamethasone on days 1, 2, and 3 of life, respectively. The animals in the control group received equal volumes (10 μl/g body wt) sterile pyrogen-free saline. Temperature and humidity were kept constant, and the rats had free access to food and water. An artificial 12-h light-dark cycle was used. The rats were weaned on day 21 and studied at 8, 50, or 80 wk of age. In this study, principles of laboratory animal care according to NIH publication No. 86–23 (revised 1985) were followed. Animal care was in accordance with Dutch national laws and the study protocol was approved by the Animal Research Committee of the University of Leiden.

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Animal Preparation

The rats were sedated by inhalation of a mixture of halothane (4%) and oxygen. Subsequently, general anesthesia was initiated by ip injection of a fentanyl-fluanison-midazolam mixture (FFM). The FFM mixture consisted of 2 parts Hynnorm (Janssen Pharmaceutica, Tilburg, The Netherlands; 0.315 mg/ml fentanyl + 10 mg/ml fluanison), 1 part Dormicum (Roche, Mijdrecht, The Netherlands; 5 mg/ml midazolam) and 1 part saline. This mixture was administered in a dose of 0.4 ml/100 g body wt. Supplemental ip injections (one-third of the initial dose) were provided if necessary so that the animals remained unresponsive to tail pinch by forceps. The animals were placed on a controlled warming pad to keep body temperature constant. A tracheostomy was performed, a cannula was inserted and connected to a pressure-controlled respirator, and ventilation was started with an air/oxygen mixture (FIO2 pressure-controlled). A catheter was introduced and positioned into the left ventricle (LV), guided by a conductance catheter (SPR-878, Millar Instruments, Houston, TX). Via the carotid artery a miniaturized combined pressure-conductance catheter (SPR-878, Millar Instruments, Houston, TX) was introduced and positioned into the left ventricle (LV), guided by on-line pressure and volume signals (14). The abdomen was opened via a small incision just below the diaphragm to enable temporary preload reductions by directly compressing the inferior vena cava with a cotton-tipped stick. The pressure-conductance catheter was connected to a Sigma-SA signal processor (CD Leycom, Zoetermeer, The Netherlands) on the ascending aorta after opening the thorax at the end of the experiment via a midsternal incision; slope factor α was calculated as cardiac output determined by conductance divided by cardiac output determined by aortic flow.

Heart Preparation

After the hemodynamic measurements, a cannula was inserted retrogradely into the abdominal aorta to allow external perfusion of the heart. The hearts were arrested in diastole by slowly infusing 1 ml 0.1 M cadmium chloride via a needle introduced in the apex of the left ventricle. Subsequently, the right atrium was cut to allow drainage, and external perfusion via the aortic cannula was started via a reservoir at ~70 cm height. A mixture of saline and nitroprusside (0.1 mg/ml) was infused for 3 min to achieve coronary vasodilatation followed by 3 min perfusion with formalin solution (2%). The hearts were then excised and immersion-fixed in phosphate-buffered formalin 4%. After at least 48 h of fixation, any remaining extracardiac structures and the atria were carefully removed from the hearts, and ventricular weight was determined.

Statistical Analysis

We performed a two-way ANOVA via a linear mixed-effects model with uncorrelated terms to account for the effects of age (8, 50, or 80 wk) and the effects of treatment (saline, dexamethasone). If the model indicated significant effects, appropriate contrasts were selected to determine significant differences between group means (SPSS version 12.0; SPSS, Chicago, IL). All data are presented as mean ± SD. A probability value P < 0.05 was considered statistically significant.

RESULTS

Experiments were performed in dexamethasone- and saline-treated male rats in three age groups: the 8-wk-old group (8wk; saline: n = 12, dexamethasone: n = 10); the 50-wk-old group (50wk; saline: n = 18, dexamethasone: n = 18), and the 80-wk-old group (80wk; saline: n = 14, dexamethasone: n = 14).

Heart and Body Weights

Anatomical data for dexamethasone- and saline-treated rats in the three groups are summarized in Table 1. In the 8wk group, body weight, ventricular weight, and the ventricular-to-body weight ratio were all significantly lower in dexamethasone-treated rats (P < 0.05). No significant differences between dexamethasone- and saline-treated rats were found in the 50wk group. In the 80wk group however, the dexamethasone-treated animals again showed a significantly reduced body weight (P < 0.005) and ventricular weight (P < 0.01), but no difference in the ventricular-to-body weight ratio.

Hemodynamics

Hemodynamic indices for the three age groups are listed in Table 1. In the 8wk group no significant differences were...
found between saline- and dexamethasone-treated rats. In the 50wk group, ejection fraction was decreased in the dexamethasone-treated rats, and the ESPVR and PRSW relations were shifted significantly towards larger volumes, as indicated by the increased ESINT and decreased SWINT, respectively. These changes reflect a decrease in systolic function. In the 80wk group these differences were still present (see Fig. 1), whereas the increase in end-systolic volume also reached statistical significance, and end-diastolic volume showed a clear tendency to increase ($P = 0.091$). Despite the reductions in systolic function in the dexamethasone-treated rats, cardiac pump function was maintained in all age groups, as shown by the unchanged cardiac output and stroke work. Diastolic function was also not significantly affected.

**DISCUSSION**

Short-term adverse cardiovascular side effects of neonatal dexamethasone treatment, including hypertension and hypertrophic cardiomyopathy, are well documented both in experimental studies and in humans (7, 8, 17, 28, 29, 35, 39). However, possible detrimental long-term effects have not been studied in detail. Clinical data, in this respect, are not available, since neonatal dexamethasone treatment started in the early

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**Table 1. Weights and hemodynamic indices in 8-, 50-, and 80-wk-old saline-treated and dexamethasone-treated rats**

<table>
<thead>
<tr>
<th></th>
<th>8 weeks (n = 12)</th>
<th>50 weeks (n = 10)</th>
<th>P</th>
<th>80 weeks (n = 14)</th>
<th>Dexa (n = 14)</th>
<th>P</th>
<th>ANOVA Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight</strong></td>
<td></td>
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<tr>
<td>BW, g</td>
<td>263 ± 33</td>
<td>240 ± 11</td>
<td>&lt;0.05</td>
<td>537 ± 39</td>
<td>528 ± 42</td>
<td>NS</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>VW, g</td>
<td>0.97 ± 0.11</td>
<td>0.83 ± 0.07</td>
<td>&lt;0.005</td>
<td>1.29 ± 0.10</td>
<td>1.22 ± 0.14</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SW/BW, mg/g</td>
<td>3.69 ± 0.21</td>
<td>3.45 ± 0.25</td>
<td>&lt;0.05</td>
<td>2.39 ± 0.30</td>
<td>2.32 ± 0.22</td>
<td>0.01</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Pump function</strong></td>
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<tr>
<td>HR, beats/min</td>
<td>443 ± 22</td>
<td>424 ± 32</td>
<td>NS</td>
<td>415 ± 25</td>
<td>419 ± 30</td>
<td>NS</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>70 ± 13</td>
<td>64 ± 10</td>
<td>NS</td>
<td>101 ± 21</td>
<td>98 ± 24</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SW, ml/mMgH</td>
<td>13.8 ± 4.0</td>
<td>11.8 ± 1.6</td>
<td>&lt;0.05</td>
<td>15.9 ± 4.3</td>
<td>14.7 ± 4.5</td>
<td>&lt;0.01</td>
<td>&lt;0.004</td>
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<tr>
<td><strong>Systolic function</strong></td>
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<tr>
<td>ESV, µl</td>
<td>96 ± 44</td>
<td>101 ± 72</td>
<td>NS</td>
<td>320 ± 108</td>
<td>383 ± 114</td>
<td>&lt;0.05</td>
<td>&lt;0.009</td>
</tr>
<tr>
<td>ESP, mmHg</td>
<td>82 ± 14</td>
<td>79 ± 17</td>
<td>NS</td>
<td>60 ± 13</td>
<td>57 ± 15</td>
<td>NS</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>EF, %</td>
<td>62 ± 11</td>
<td>61 ± 14</td>
<td>&lt;0.05</td>
<td>43 ± 6</td>
<td>38 ± 6</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>dP/dtMAX, mmHg/ms</td>
<td>12.6 ± 2.0</td>
<td>10.2 ± 3.0</td>
<td>&lt;0.05</td>
<td>10.2 ± 2.8</td>
<td>9.4 ± 3.4</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ees, mmHg/µl</td>
<td>0.21 ± 0.08</td>
<td>0.26 ± 0.08</td>
<td>&lt;0.05</td>
<td>0.15 ± 0.05</td>
<td>0.16 ± 0.06</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
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<tr>
<td>SWINT, µl/mmHg</td>
<td>82 ± 92</td>
<td>92 ± 127</td>
<td>NS</td>
<td>301 ± 143</td>
<td>405 ± 140</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
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<tr>
<td>SWINT, ml/mMgH</td>
<td>13.7 ± 3.3</td>
<td>12.1 ± 4.5</td>
<td>&lt;0.05</td>
<td>19.2 ± 7.2</td>
<td>13.0 ± 5.4</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Diastolic function</strong></td>
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<tr>
<td>EDV, µl</td>
<td>242 ± 62</td>
<td>236 ± 85</td>
<td>NS</td>
<td>556 ± 156</td>
<td>614 ± 145</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>4.6 ± 1.6</td>
<td>4.2 ± 1.8</td>
<td>NS</td>
<td>4.5 ± 1.4</td>
<td>4.2 ± 1.6</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>dP/dt/diMax, mmHg/ms</td>
<td>6.6 ± 1.7</td>
<td>5.9 ± 2.1</td>
<td>&lt;0.05</td>
<td>4.6 ± 0.8</td>
<td>4.0 ± 1.3</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>τ, ms</td>
<td>10.6 ± 1.9</td>
<td>12.1 ± 2.6</td>
<td>&lt;0.05</td>
<td>11.3 ± 3.2</td>
<td>12.0 ± 2.9</td>
<td>NS</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Ees, mmHg/µl</td>
<td>0.027 ± 0.017</td>
<td>0.029 ± 0.012</td>
<td>NS</td>
<td>0.019 ± 0.011</td>
<td>0.021 ± 0.012</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SWINT, µl/mmHg</td>
<td>4.0 ± 2.1</td>
<td>3.1 ± 3.3</td>
<td>NS</td>
<td>6.2 ± 3.3</td>
<td>4.5 ± 4.0</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SD. Dexa, dexamethasone; BW, body weight; VW, ventricular weight; HR, heart rate; CO, cardiac output; SW, stroke work; ESV, end-systolic volume; ESP, end-systolic pressure; EF, ejection fraction; Ees, end-systolic elastance; SWINT, volume intercept of end-systolic pressure-volume relation (calculated at mean ESP: 81 mmHg for 8 wk, 58 mmHg for 50 wk, 64 mmHg for 80 wk); SWINT, slope of preload recruitable stroke work relation; SWINT, intercept of PRSW (calculated at mean EDV: 239 µl for 8 wk, 585 µl for 50 wk, 529 µl for 80 wk); EDV, end-diastolic volume; EDP, end-diastolic pressure; τ, time constant of isovolumic relaxation; Ees, end-diastolic elastance; SWINT, pressure intercept of end-diastolic pressure-volume relation (calculated at mean EDV).
90s. However, a substantial patient cohort is currently reaching adulthood. Our previous hemodynamic studies in this rat model only covered the early stage (4 wk), whereas from adult and elderly stages mainly histopathological data and only very limited hemodynamic data have been reported. The studies in 4-wk-old prepubertal rats revealed reduced heart weight and depressed systolic function with compensatory dilatation and maintained cardiac output (4). Histopathological studies indicated that these cardiovascular effects were presumably due to suppression of cardiomyocyte mitosis during dexamethasone treatment, resulting in a significantly reduced number of cardiomyocytes (10). In addition, recent data indicated a reduced life span in rats treated neonatally with dexamethasone (20). Our previous hemodynamic data in adult animals was limited to blood pressure and heart rate recordings by telemetry at 3 and 11 mo of age (13 and 48 wk, respectively). These data indicated a higher systolic blood pressure in the dexamethasone-treated rats (+6% at 3 mo and +10% at 11 mo), but no differences in heart rate, diastolic pressure, or mean arterial blood pressure (20).

The present longitudinal study investigated cardiovascular function during life span in 8-, 50-, and 80-wk-old rats. Our findings indicated that at 8 wk the rats still had reduced heart and body weights, but much less pronounced than at 4 wk. Our previous studies documented 27% reduction in ventricular weight and 12% reduction in body weight at 4 wk, whereas in the present study we found, respectively, 14% and 9% reduction at 8 wk. At 50 wk the ventricular and body weights were fully normalized, but at 80 wk the dexamethasone-treated rats again showed significantly reduced ventricular and body weights by 12% and 10%, respectively. In line with these findings, the cardiac function studies revealed that the systolic dysfunction previously found at 4 wk was normalized at 8 wk, but reappeared at 50 wk and, more pronouncedly, at 80 wk. In all age groups global pump function quantified by cardiac output and stroke work was maintained. However, the animals with depressed systolic function showed evidence of compensatory cardiac dilatation, indicating that these animals used their Frank-Starling mechanism to maintain normal cardiac output, although the increase in end-diastolic volume did not reach statistical significance (P = 0.091).

Thus our study supports a previous hypothesis that neonatal dexamethasone treatment may lead to cardiovascular dysfunction later in life. However, the present data did not show a continuous decline of cardiac function, but rather indicated that the systolic dysfunction at 4 wk was followed by normalization in the adult phase and gradual development of systolic dysfunction only in the elderly animals. Importantly, these effects were not only evident from a reduced ejection fraction, but also from significant rightward shifts of the ESPVR and PRSW relationships. The positions of these relations are relatively load-independent (9, 21, 38). Markers of systolic function and the shifts therefore confirm a true decline in intrinsic ventricular function and exclude that the reduced ejection fraction merely results from, e.g., increased afterload in the dexamethasone-treated animals. The slopes of the ESPVR and PRSW were not affected; however, previous studies indicate that the positions of these relationships, particularly when determined at a physiological level, yield more sensitive and consistent indexes for detection of changes in contractile state (9, 37).

Presumably, the early effects of dexamethasone on ventricular function are gradually masked by compensatory cellular hypertrophy in the adult animals, but become manifest again in the elderly animals. We would speculate that the depressed systolic function was linked with a substantially lower number of cardiomyocytes in the dexamethasone-treated rats, resulting from suppression of proliferation during dexamethasone treatment (10). The ventricular weight measurements indicated that the lower number of cardiomyocytes was partly compensated by cellular hypertrophy in the 8-wk-old group, but fully compensated only in the 50-wk-old animals. Apparently, the development of cellular hypertrophy in this model is relatively slow and not fully completed at 8 wk. The reduced ventricular weight at 80 wk may reflect that possibilities for cellular hypertrophy become exhausted in the elderly dexamethasone-treated rats or that these animals show increased cell loss, as previously suggested, reflecting early aging.

Interestingly, in this study we did not observe increased mortality in the dexamethasone-treated rats as was seen in a previous study (20). Whereas in the earlier study 25% of dexamethasone-treated rats died prematurely before 80 wk (18 mo), none of the rats in the present study died prior to the hemodynamic studies. A possible explanation could be that the rats in the present study were housed in groups of 4–6 rats, whereas in the earlier study each rat was housed individually. Based on behavioral studies (31), solitary animals are exposed to more stress, which could have amplified the differences between dexamethasone-treated and control animals and led to reduced life span. Our current findings also do not confirm the 10% increase in systolic blood pressure previously found in adult rats (20). Besides the different housing mentioned above, this may also be related to the fact that the present study was performed in anesthetized animals in contrast to the conscious state in the previous study.

Extrapolation of the results found in this study in rats to the clinical practice of dexamethasone treatment in humans remains speculative. We used term rat pups as a model for premature human babies. With regard to brain development, a 7-day-old rat is roughly equivalent to a full-term human infant, and thus in this respect term rat pups correspond well to premature infants (13). Furthermore, at day 1–3 after birth, the period during which dexamethasone treatment was installed, the rat myocardium still shows substantial hyperplasia (23), similar to the preterm human myocardium (16, 26). Thus with respect to the transition from myocyte hyperplasia to hypertrophy, the term rat pup is also a good model for the preterm human infant. However, potential differences in the degree of maturation of neurohormonal systems, receptor mechanisms, and innervation (33) may also be of influence in the response to dexamethasone treatment. The development of these systems has been studied in the rat (30), but very few data are available for the (preterm) human situation. Thus, although species differences may be of influence, this model is frequently used, and several effects of dexamethasone treatment found in humans, including early transient hypertrophy, were also found in the rat model (11, 22, 27, 34).

We conclude that the reduced ventricular weight and systolic dysfunction previously found in 4-wk-old rats after neonatal dexamethasone treatment is transient, presumably due to compensatory cellular hypertrophy, but becomes manifest again in elderly rats. The elderly animals with depressed systolic func-
tion showed evidence of compensatory cardiac dilation, indicating that these animals have to invoke the Frank-Starling mechanism to maintain normal cardiac output. If our results are applicable to humans, a relatively large patient population is involved, and early screening and a cardiovascular follow-up program may be warranted to enable secondary prevention.

GRANTS

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REFERENCES